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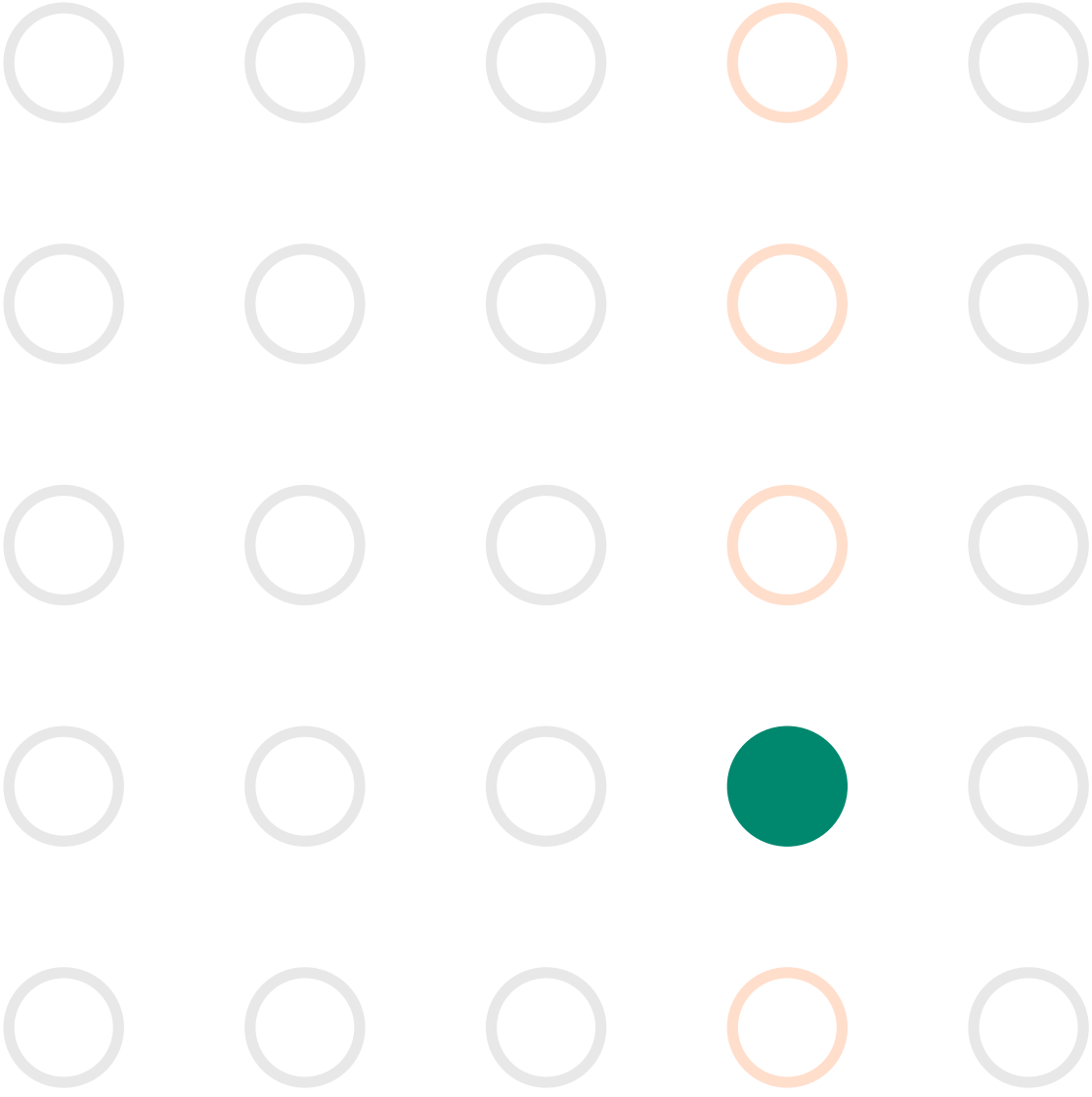
Frequently asked questions

Ni Sepharose Fast Flow
HisTrap Fast Flow
HisPrep 16/10 Fast Flow



Q When should I use the Ni Sepharose™ 6 Fast Flow instead of Ni Sepharose High Performance?

A The new Ni Sepharose 6 Fast Flow and Ni Sepharose High Performance are optimized for the purification of Histidine-tagged proteins and the media are pre-charged with Ni^{2+}.

Ni Sepharose 6 Fast Flow is designed for fast, reliable scale-up of Histidine-tagged protein purification. The medium is also well suited for batch/gravity flow purification and multi-well plate screening.

Ni Sepharose High Performance is optimized for high performance purification of Histidine-tagged proteins with a chromatography system, such as an ÄKTA system from Cytiva.

Q What is the protein binding capacity of Ni Sepharose 6 Fast Flow?

A Dynamic binding capacity: Approximately 40 mg $(His)_6$-tagged protein/mL medium

Dynamic binding capacity conditions:

Sample:	1 mg/mL $(His)_6$-tagged pure protein (M_r 43 000) in binding buffer ($QB_{10\%}$ determination) or $(His)_6$-tagged protein (M_r 28 000) bound from *E. coli* extract
Column volume:	0.25 mL or 1 mL
Flow rate:	0.25 mL/min or 1 mL/min
Binding buffer:	20 mM sodium phosphate, 0.5 M NaCl, 5 mM imidazole, pH 7.4
Elution buffer:	20 mM sodium phosphate, 0.5 M NaCl, 500 mM imidazole, pH 7.4

Note: Dynamic binding capacity is protein dependent.

Q What are the general running conditions recommended?

A **Recommended starting conditions:**

Binding buffer:	20 mM sodium phosphate, 0.5 M NaCl, 20–40 mM imidazole, pH 7.4
Elution buffer:	20 mM sodium phosphate, 0.5 M NaCl, 500 mM imidazole, pH 7.4

Note: The concentration of imidazole that will give optimal purification results (in terms of purity and yield) is protein-dependent, and is usually slightly higher for Ni Sepharose 6 Fast Flow than for similar IMAC media on the market. This can be useful during the washing step as a higher concentration of imidazole in the wash buffer may wash out contaminants that have bound to the medium. This can result in elution of a more pure target protein.

Higher concentration of imidazole will not cause any problems in terms of increased baseline absorbance if a high purity imidazole is used, such imidazole gives essentially no absorbance at 280 nm.

Q Will the high capacity of the Ni Sepharose 6 Fast Flow result in higher non-specific binding?

A The non-specific binding of proteins is a result of several factors. Other proteins will only bind more to Ni Sepharose 6 Fast Flow if these non-specific proteins are interacting directly with the Ni^{2+} or the metal chelating ligand. In addition, non-specific proteins can potentially be washed off at higher concentrations of imidazole, while keeping the target protein bound to the medium.

Q With the higher binding capacity, do you have to use harsh conditions to remove the target protein?

A Harsh conditions are not necessary to remove most target proteins. The proteins tested are all eluted below 500 mM imidazole; frequently between 100-300 mM imidazole. The protein is efficiently eluted from the column, resulting in high yields.

Q How many times can you run the Ni Sepharose 6 Fast Flow while maintaining the same capacity?

A This is sample dependent. Reusing Ni Sepharose 6 Fast Flow depends on the nature of the sample. To prevent cross-contamination, only reuse the column when purifying identical Histidine-tagged proteins.

Q How do you measure the amount of Ni^{2+} in the eluent?

A This quantitative measurement assay is as follows:

The medium is run with 10 column volumes of a buffer with pH 4.0 and then the Ni^{2+} still bound on the column is measured spectroscopically and compared with the amount Ni^{2+} loaded.

Q Can you strip and regenerate the Ni Sepharose 6 Fast Flow medium?

A The Ni Sepharose 6 Fast Flow can be stripped with 50 mM EDTA in 20 mM sodium phosphate, 0.5 M NaCl, pH 7.4 and recharged with Ni^{2+} or other divalent cations, such as Cu^{2+}, Co^{2+}, Zi^{2+}, etc.

Q How can I strip and recharge the medium?

A Recommended stripping buffer: 20 mM sodium phosphate, 0.5 M NaCl, 50 mM EDTA, pH 7.4.

1. Strip the column by washing with 5–10 column volumes of stripping buffer.
2. Wash with 5–10 column volumes of binding buffer.
3. Wash with 5–10 column volumes of distilled water.
4. Re-charge the column.

To recharge the water-washed column:

1. Load 0.5 column volumes of 0.1 M metal salt solution in distilled water on the column. Metal chloride and sulfate salts, e.g. 0.1 M $NiSO_4$, are commonly used.
2. Wash with 5 column volumes of distilled water.
3. Wash with 5 column volumes of binding buffer.

Note: It is important to wash with binding buffer as the last step to get pH correct.

Q How is the Ni^{2+} leakage at low pH?

A At pH 4, the loss of Ni^{2+} is < 5% on Ni Sepharose 6 Fast Flow (reproducible) compared to average 9% for Ni-NTA Superflow from Qiagen (wide batch to batch variations).

Note: Loss of metal ions is more pronounced at lower pH.

Q My target protein is extremely sensitive to Ni^{2+}. How do I reduce the Ni^{2+} leakage to a real minimum (below < 5%)?

A Leakage of Ni^{2+} from Ni Sepharose 6 fast Flow is low under all conditions and resistance towards reducing agents is thus high.

Note: If using buffers/sample including reducing agents it is recommended to perform a blank run *without* reducing agents in the buffers before loading sample (to remove any weakly bound Ni^{2+} ions):

Blank run: Use binding and elution buffer *without* reducing agents

1. Wash the column with 5 column volumes of distilled water.
2. Wash with 5 column volumes of binding buffer.
3. Wash with 5 column volumes of elution buffer.
4. Equilibrate with 10 column volumes of binding buffer.

Q What different additives can I use together with Ni Sepharose 6 Fast Flow, HisPrep™ FF 16/10 and HisTrap™ FF?

A Tests performed in our laboratories show that Ni Sepharose 6 fast Flow is compatible with the following compounds at the concentrations given.

Reducing agents	5 mM DTE	
	5 mM DTT	
	20 mM β-mercaptoethanol	
	5 mM TCEP	
	10 mM reduced glutathione	
Denaturing agents	8 M urea*	
	6 M guanidine hydrochloride*	
Detergents	2% Triton™ X-100	Non-ionic detergent
	2% Tween™ 20	Non-ionic detergent
	2% NP-40	Non-ionic detergent
	2% cholate	Anionic Detergent
	1% CHAPS	Zwitterionic Detergent
Additives	500 mM imidazole	
	20% ethanol	
	50% glycerol	
	100 mM Na_2SO_4	
	1.5 M NaCl	
	1 mM EDTA**	
	60 mM citrate**	
Buffer solutions	50 mM sodium phosphate, pH 7.4	
	100 mM Tris-HCl, pH 7.4	
	100 mM Tris-acetate, pH 7.4	
	100 mM HEPES, pH 7.4	
	100 mM MOPS, pH 7.4	
	100 mM sodium acetate, pH 4*	

* Tested for 1 week at 40°C

** Generally, chelating agent should be used with caution (and only in the sample, not in buffers). Any metal ion stripping may be counteracted by addition of a small excess of $MgCl_2$ before centrifugation or filtration of the sample. Note that stripping effects may vary with applied sample volume.

Q How do I remove imidazole from my target protein after elution?

A If imidazole needs to be removed from the protein, use HiTrap™ Desalting 5 mL (can be coupled in series to increase the sample volume), a PD-10 column, or HiPrep™ 26/10 Desalting, depending on the sample volume.

Q Do you see an improved purity (less non-specific binding) with high concentrations of NaCl?

A In most cases, concentration of ions, imidazole, and other additives in the binding and wash buffers will influence the level of non-specific binding. Higher concentrations of NaCl (500 mM) generally reduce the level of non-specific binding.

Q How does DTT affect the performance of Ni Sepharose 6 Fast Flow in comparison to nickel media from other tested suppliers?

A DTT has a drastic effect on the color and performance of nickel medium from other tested suppliers, so DTT is generally avoided.

For Ni Sepharose 6 Fast Flow, however, DTT has been tested up to 5 mM and does not affect the binding capacity or nickel ion leakage of the Ni Sepharose 6 Fast Flow. There is no effect on the performance of the medium, although color changes can be evident over 3 mM DTT. *Therefore, it is essential to perform a blank run before loading sample/buffers including DTT (and other reducing agents).*

Blank run: Use binding and elution buffer *without* reducing agents

1. Wash the column with 5 column volumes of distilled water.
2. Wash with 5 column volumes of binding buffer.
3. Wash with 5 column volumes of elution buffer.
4. Equilibrate with 10 column volumes of binding buffer.

Q Is it possible to use high concentrations of Tris-HCl buffer and how does this compatibility to Tris buffers compare to other suppliers?

A These are the Tris buffers systems that have been tested and work well.

50 mM Tris-HCl, pH 7.4
100 mM Tris-HCl, pH 7.4
100 mM Tris-acetate, pH 7.4

Tris buffers in high concentrations can adversely affect the binding of Histidine-tagged target proteins to nickel medium from other suppliers; therefore, lower concentrations of Tris buffers (less than 50 mM) or phosphate buffers are generally used.

Q How can I clean Ni Sepharose 6 Fast Flow?

A **Note:** Strip off the metal ions before cleaning (to prevent precipitation of metal salt)

- To remove precipitated proteins, hydrophobically bound proteins and lipoproteins:
  1. Wash the column with 1 M NaOH, contact time usually 1–2 hours (12 hours or more for endotoxin removal).
  2 Wash the column with approximately 10 column volumes of binding buffer.
  3. Wash the column with 5–10 column volumes of distilled water.

- To remove strongly hydrophobically bound proteins, lipoproteins and lipids:
  1. Wash the column with 5–10 column volumes 30% isopropanol for about 15–20 minutes.
  2. Wash the column with approximately 10 column volumes of distilled water.
  3. Re-charge the medium or wash with 5 column volumes 20% ethanol for storage.

Alternatively:

1. Wash the column with 2 column volumes of detergent in a basic or acidic solution. Use, for example, 0.1–0.5% nonionic detergent in 0.1 M acetic acid, contact time 1–2 hours.
2. After treatment, always remove residual detergent by washing with 5 column volumes of 70% ethanol.
3. Wash the column with approximately 10 column volumes of distilled water.
4. Re-charge the medium or wash with 5 column volumes 20% ethanol for storage.

- To remove ionically bound proteins:
  1. Washing the column with 1.5 M NaCl solution, contact time 10–15 minutes.
  2. Wash the column with approximately 10 column volumes of distilled water.
  3. Re-charge the medium or wash with 5 column volumes 20% ethanol for storage.

Q Is it possible to use the Ni Sepharose 6 Fast Flow by gravity or in 96 or 384 well multi-well plates for screening?

A Yes, the performance by gravity flow has been tested. The Ni Sepharose 6 Fast Flow can be used to fill empty gravity columns and multi-well plates.

Q Do the HisTrap FF columns have Valco fittings?

A Yes. This makes it possible to put these columns directly into ÄKTAdesign without any extra connectors.

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